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ACHENE MICROMORPHOLOGY OF THE *CAREX NIGROMARGINATA* COMPLEX (SECTION *ACROCYSTIS*, CYPERACEAE)

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ABSTRACT

Principal component and cluster analyses, in addition to individual achene micromorphological characters, divide the *Carex nigromarginata* complex into two groups: achenes of *C. nigromarginata, C. floridana* and *C. peckii* have cell central bodies with concave sides while the remaining three taxa have cell central bodies with convex sides. This evidence supports the reduction of *C. artitecta* and *C. physorhyncha* to varieties of *C. emmonsii* (as *C. emmonsii* var. *muhlenbergii, C. emmonsii* var. *australis* and *C. emmonsii* var. *emmonsii,* respectively). The six taxa can only be distinguished by significant differences between the achene characters; achene micromorphological data are most useful in dividing the complex into sets of taxa.

Key Words: *Carex, Carex nigromarginata, Acrocystis, Montanae,* Cyperaceae, micromorphology, SEM, Angiosperm systematics, eastern North America

INTRODUCTION

The *Carex nigromarginata* Schwein. complex consists of six closely related taxa within section *Acrocystis* Dumort. (= *Montanae* (Fries) Carey). Mackenzie (1935) treated these taxa as six distinct species: *C. nigromarginata, C. floridana* Schwein., *C. peckii* Howe ex Peck, *C. emmonsii* Dewey ex Torrey (as *C. albicans* Willd. ex Sprengel), *C. artitecta* Mackenzie and *C. physorhyncha* Liebm. ex Steudel. Radford et al. (1968) and Lahham (1980, Ph.D. dissertation, Penn. State Univ.), however, included *C. floridana* as a variety of *C. nigromarginata.* Gleason and Cronquist (1963) and Scoggan (1978) treated *C. peckii, C. emmonsii* and *C. artitecta* as varieties of *C. nigromarginata.* Voss (1972) indicated that *C. artitecta and C. emmonsii* are "distinguished only with great difficulty."

The above treatments were all essentially macromorphological in nature and other types of data have been employed in other groups of *Carex* to help clarify both infra- and interspecific re-

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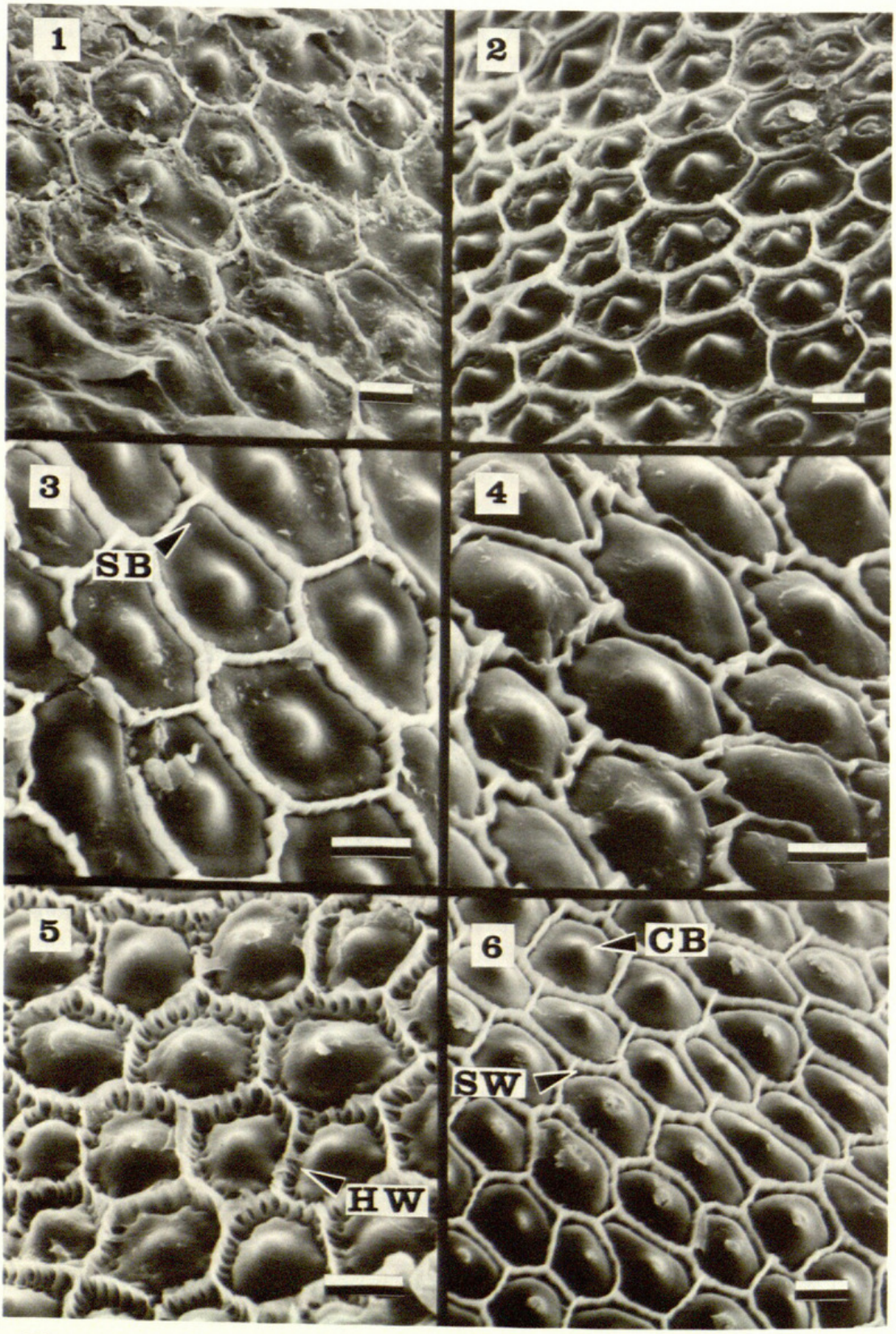
lationships. Other types of data also have been helpful within this complex. Rettig (1988, Ph.D. dissertation, Univ. Georgia, Athens; 1989) treated *C. artitecta* and *C. physorhyncha* as varieties of *C. emmonsii* (*C. emmonsii* var. *muhlenbergii* (A. Gray) J. Rettig and *C. emmonsii* var. *australis* (L. Bailey) J. Rettig, respectively) based on phenetic analyses of macromorphological data, flavonoids and achene micromorphology.

Achene micromorphology has proved taxonomically useful in numerous species groups in *Carex* (e.g., Walter, 1975; Toivonen and Timonen, 1976; Timonen and Toivonen, 1979; Toivonen, 1980; Hoshino, 1984; Menapace and Wujek, 1985; Standley, 1985, 1986, 1987a, 1987b; Wujek and Menapace, 1986; Bruederle et al., 1989). Selected micromorphological characters have also been studied in some North American members of section *Acrocystis*. Lahham (1980, op. cit.) investigated epidermal peels, internal anatomy of culms and leaf blades, and surfaces of pollen grains and achenes. Of these characters, Lahham concluded that only achene surfaces were useful in distinguishing taxa.

Most of the above studies have been subjective comparisons of achenes between taxa. Menapace et al. (1986) and Menapace and Wujek (1987) made the first attempts at an objective approach to examining the surface features of *Carex* achenes. In each study, a data matrix and phenogram was constructed by scoring micrographs with characters that "could be scored more or less unequivocally for each species" (Menapace et al., 1986). Preliminary examination of achene micrographs within the *C. nigromarginata* complex (Figures 1–6) revealed that variation between the achenes of a single taxon and even among the cells of a single achene would make it difficult to unequivocally assign a character state to a taxon or even to a single achene. An attempt was made to overcome this problem by utilizing a method that does not require the entire taxon to be scored unequivocally. Variation within a taxon was taken into account by scoring characters on randomly chosen cells on a single achene.

METHODS AND MATERIALS

Plant materials used are cited in Table 1. Specimens from each taxon were selected to represent a wide range of geographical and macromorphological variation. Achenes were treated with acetic acid for 24 hr., placed in a bath-type ultrasonic cleaner for up to



Figures 1–6. Representative scanning electron micrographs of the achene surface in the *Carex nigromarginata* complex. **1.** *C. nigromarginata* (*Rettig 1430*). **2.** *C. floridana* (*Rettig 1514*). **3.** *C. peckii* (*Cody 6930*). **4.** *C. emmonsii* var. *emmonsii* (*Rettig 1030*). **5.** *C. emmonsii* var. *muhlenbergii* (*Morton 7132*). **6.** *C. emmonsii* var. *australis* (*Rettig 1525*). CB = central body, HW = honeycombed anticlinal wall, SB = satellite body, SW = sinuous anticlinal wall. Scale bars = 10 μ.

Table 1. Origin and accession number of collections of the *Carex nigromarginata* complex sampled.

Carex nigromarginata. **Alabama:** Tuscaloosa Co., *Rettig 1447* (GA). **Georgia:** Hancock Co., *Rettig 1430* (GA); Habersham Co., *Manhart 212* (GA); Union Co., *Rettig 1456* (GA).

Carex floridana. **North Carolina:** Bladen Co., *Rettig 1514* (GA); Brunswick Co., *Godfrey 49005* (GA).

Carex peckii. **New York:** Essex Co., *House 8059* (NY). CANADA. **Alberta:** *Cody 6930* (GH). **British Columbia:** *Calder 17030* (GA).

Carex emmonsii var. *emmonsii.* **North Carolina:** Madison Co., *Rettig 1030* (GA). **Pennsylvania**: Centre Co., *Wahl 977* (GH); Tioga Co., *Rettig 1330* (GA); Warren Co., *Rettig 1343* (GA). **Tennessee:** Johnson Co., *Rettig 1538* (GA). **Virginia:** James City Co., *Rettig 1530* (GA).

Carex emmonsii var. *muhlenbergii.* **Arkansas:** Polk Co., *Rettig 1483* (GA); Pope Co., *Rettig 1490* (GA). **New Jersey:** Warren Co., *Morton 7132* (NY). **North Carolina:** Transylvania Co., *Rettig 1555* (GA).

Carex emmonsii var. *australis.* **Alabama:** Henry Co., *Rettig 1440* (GA); Marshal Co., *Kral 34196* (GA). **Arkansas:** St. Francis Co., *Rettig 1497* (GA). **Florida:** Liberty Co., *Rettig 1224* (GA). **Mississippi:** Calhoun Co., *Rettig 1452* (GA); Washington Co., *Rettig 1455* (GA). **Texas:** Shelby Co., *Rettig 1459* (GA). **Virginia:** Brunswick Co., *Rettig 1526* (GA). MEXICO. **Chapingo:** *Koch 7827* (MO).

2 hr. or a probe-type sonifier for up to 15 min. to remove the outer periclinal wall, air-dried and then sputter-coated in a Hummer V Sputter Coater to give a coating of approximately 20 μ in thickness. Upper shoulders of achenes were photographed using a JOEL scanning electron microscope at an accelerating voltage of 15 kV.

A data matrix was constructed using 10 characters (8 binary, 2 quantitative) that could be scored for each cell on every achene (Table 2). Five randomly chosen cells were scored for each achene and means ($\bar{Y}$) were then calculated to give a single score for each character for every achene. To identify characters contributing most to differences between taxa, a principal component (PC) analysis was performed using standardized data (Sneath and Sokal, 1973), and a correlation matrix was generated to determine which characters were significantly correlated with the PC's. General linear modeling was performed to determine which characters were significantly different between the taxa. Standardized data were used to calculate pair-wise similarity-dissimilarity matrices using euclidian distances, and phenograms were prepared using average linkage clustering (UPGMA). Alpha level for all statistical procedures was .05 unless otherwise specified. Cluster analysis

Table 2. Characters used in the achene micromorphological phenetic analysis.

Acronym	Derivation
APEX	Apex shape of central body—rounded vs. pointed
APPRESS	Margins of platform appressed to platforms of adjacent cells (Figure 2)—vs. nonappressed (Figure 4)
CBSIDE	Sides of central body/platform—convex vs. concave (sunken below surface)
CENBOD	Central body (bump on top of platform)—present or absent
LENGTH	Maximum length of cell at widest point
NUMWALL	Number of anticlinal walls
PLATFM	Corners of platform (raised portion of central portion of cell)—round vs. angular
SATBOD	Satellite bodies (raised bumps) at margins of platform—present (Figure 3) or absent
SHPWALL	Shape of anticlinal walls—curved vs. linear
TYPWALL	Type of anticlinal walls—solid vs. honeycombed (Figure 5) or sinuous (Figure 6)

was performed via SPSS/PC+ (Norusis, 1986); other analyses were performed with SAS Version 6 (1987).

RESULTS AND DISCUSSION

Representative scanning electron micrographs of the six taxa in the complex are shown in Figures 1–6. Figure 1 shows the outer periclinal wall partially removed to expose the diagnostic features of the surface. The complex in general is characterized by fewer diagnostic characters than are used to distinguish some other species of *Carex* (e.g., Hoshino, 1984).

The complex can be divided into two groups based on the shapes of the sides of the central body/platforms. This character (CBSIDE) is constant for all cells of all achenes of all taxa examined. Achenes of *Carex nigromarginata, C. floridana* and *C. peckii* (Figures 1–3) are characterized by a central body with concave sides compared to a central body with convex sides in *C. emmonsii* s.l. (Figures 4–6).

This division of the complex into two groups is supported by multivariate analyses of the characters. A plot of the first two PC's (accounting for 37.1% and 17.3% of the variation, respectively) also divides the complex into two sets of taxa: *Carex nigromarginata, C. floridana* and *C. peckii* on the positive end of

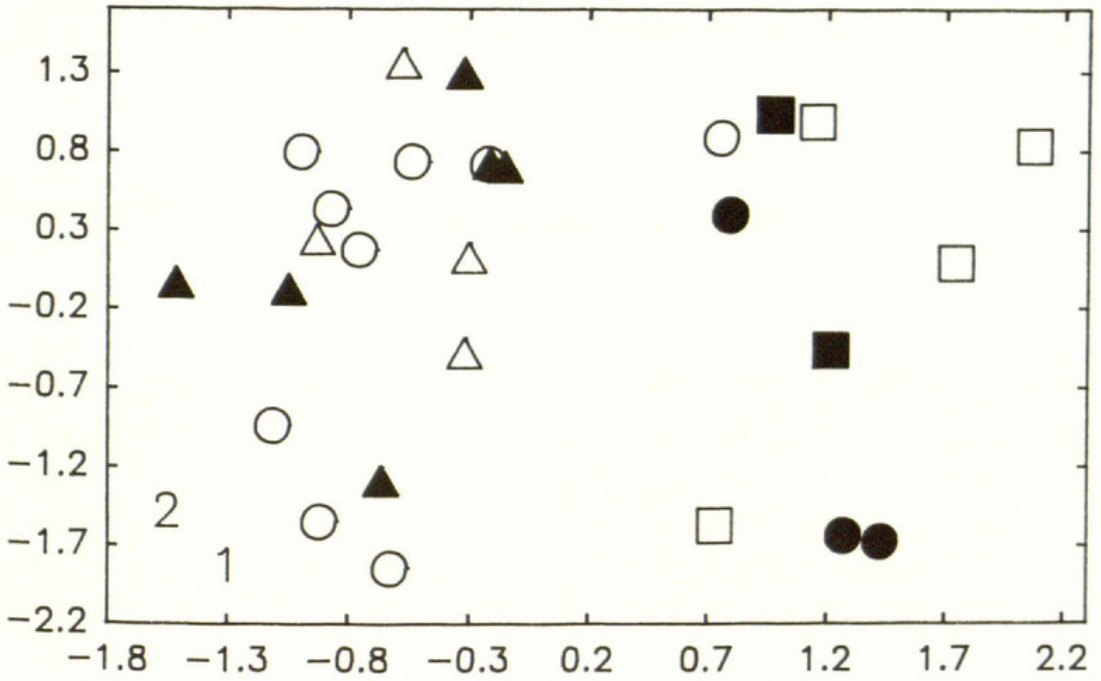


Figure 7. Principal component ordination of the *Carex nigromarginata* complex specimens. *C. nigromarginata* (■); *C. floridana* (□); *C. peckii* (●); *C. emmonsii* var. *emmonsii* (▲); *C. emmonsii* var. *muhlenbergii* (△); *C. emmonsii* var. *australis* (○). Characters correlated ($P \leq .001$) to the PC's were (acronyms according to Table 2): PC1—CBSIDE, APPRESS, PLATFM, SATBOD, and LENGTH; PC2—NUMWALL and SHPWALL.

PC1 and *C. emmonsii* s.l. toward the negative end (Figure 7). Only one specimen of *C. emmonsii* var. *australis* (*Koch 7827*) is not grouped with the other specimens of *C. emmonsii* s.l. possibly due to the presence of satellite bodies on some cells. *Koch 7827* is correctly identified based on macromorphological characters. Cluster analysis (Figure 8) using the qualitative characters also divides the complex into two sets of specimens: the upper cluster contains all of the specimens of *C. nigromarginata, C. floridana* and *C. peckii*; the lower cluster contains all specimens of *C. emmonsii* s.l., including *Koch 7827*.

Segregation of the complex into two subgroups is supported by macromorphological data. Principal component, cluster and discriminant function analyses of 26 macromorphological characters also divide the complex into two sets of taxa corresponding to *C. emmonsii* s.l. and the three remaining taxa (Rettig, 1988, op. cit.). Two of these macromorphological characters (achene body length and perigynium width) also divide the complex into two sets of taxa. Additional evidence comes from flavonoid data: *C. emmonsii* s.l. lacks tricin compounds present in the remaining taxa

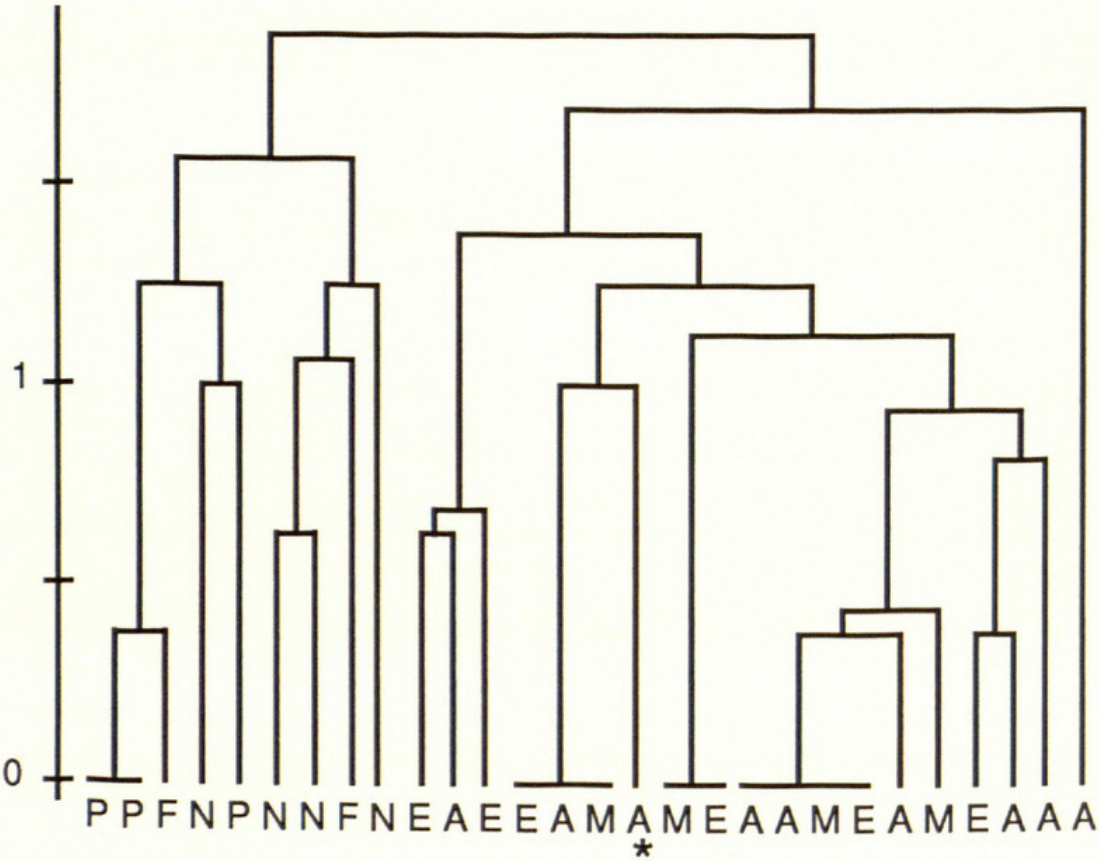


Figure 8. Phenogram for the *Carex nigromarginata* complex specimens using average linkage (UPGMA) with selected characters. Scale indicates euclidean distance. Asterisk = *Koch 7827*. N = *C. nigromarginata*, F = *C. floridana*, P = *C. peckii*, E = *C. emmonsii* var. *emmonsii*, M = *C. emmonsii* var. *muhlenbergii*, A = *C. emmonsii* var. *australis*.

and an unidentified compound is present in *C. emmonsii* s.l. but absent from the other taxa (Rettig and Giannasi, unpubl. data).

Neither PC nor cluster analyses of the achene micromorphological characters further divides the complex into discrete taxa which correspond to the macromorphology. Lack of separation could be due in part to the paucity of ornamentation and scoreable characters that can be used in the analyses or the variable nature of the achene surface. Most of the specimens cannot be assigned to a taxon based solely on examination of its micrograph, and thus it appears that the micromorphology of this complex may not be as useful at the interspecific level as has proven in other groups. However, an advantage of scoring characters on individual cells of an achene is that the data can be used to separate the taxa based on significant differences of the characters.

Carex nigromarginata (Figure 1) and *C. floridana* (Figure 2), which differ from each other in two characters (APPRESS,

PLATFM), significantly differ from the other four taxa by having central bodies of adjacent cells appressed. *Carex peckii* (Figure 3) can be distinguished from the other taxa (with the exception of *C. nigromarginata*) by having a significantly greater number of cells with satellite bodies around the central body. *Carex peckii* differs from *C. nigromarginata* in one character (PLATFM).

There is no single character that is constant in all cells of all achenes examined that can be used to distinguish the three varieties of *Carex emmonsii* s.l. (Figures 4–6). Therefore, a given specimen cannot be assigned to one of these three taxa with any degree of certainty. However, *C. emmonsii* var. *muhlenbergii* is significantly different from *C. emmonsii* var. *australis* based on the presence of a central body (*C. emmonsii* var. *muhlenbergii* always has one present, while some achenes of *C. emmonsii* var. *australis* usually have cells that lack a central body). *Carex emmonsii* var. *emmonsii* is significantly different from *C. emmonsii* var. *muhlenbergii* in the sinuous nature of the anticlinal walls. Anticlinal walls of *C. emmonsii* var. *muhlenbergii* are always sinuous while the achenes of *C. emmonsii* var. *emmonsii* usually have non-sinuous anticlinal walls.

Achene micromorphological data do not support the taxonomic conclusions of some previous authors. Both *Carex emmonsii* s. str. and *C. emmonsii* var. *muhlenbergii* have been treated as varieties of *C. nigromarginata.* The data presented here suggest that these two taxa, along with *C. emmonsii* var. *australis,* form a distinct set of taxa more closely related to each other than to *C. nigromarginata.*

Carex peckii and *C. floridana* also have been treated as varieties of *C. nigromarginata.* However, achene micromorphology suggests that both are distinct species. *Carex peckii* appears to be the most distinct taxon within the complex because of a unique combination of the presence of sinuous anticlinal walls and prominent satellite bodies. *Carex peckii* also appears to be the most derived taxon within this complex based on flavonoid chemistry (Rettig and Giannasi, unpubl. data). The species is characterized by a general loss of several flavones but has an increase in the diversity of remaining C-glycosylflavones. These trends are paralleled by the increased prominence of the satellite bodies on the achene surface which are more developed than those found in *C. nigromarginata.* Crins and Ball (1988) concluded that "increasing distinct satellite bodies" were a derived condition in sect. *Cerato-*

cystis Dumort. and correlated with trends in cytology, ecology, chemistry, achene shape, spike morphology and features of the perigynium epidermis. These correlations do not seem to occur within the *C. nigromarginata* complex as a whole. *Carex floridana* also appears to be more derived than *C. nigromarginata* based on flavonoid chemistry (Rettig, 1988, op. cit.; Rettig and Giannasi, unpubl. data) but achenes of *C. floridana* do not possess the number or development of satellite bodies of either *C. nigromarginata* or *C. peckii.*

In conclusion, achene micromorphological data provide some evidence for recognition of six distinct taxa within the *Carex nigromarginata* complex based on significance differences in the individual characters. The major value of this approach is that multivariate analysis of these characters on each achene divided the complex into two sets of taxa providing information on relationships within the complex that would otherwise not be apparent.

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